

**WEST****End of Result Set**

Generate Collection

Print

L1: Entry 1 of 1

File: USPT

Feb 8, 2000

US-PAT-NO: 6022730

DOCUMENT-IDENTIFIER: US 6022730 A

TITLE: Methods for the isolation of bacteria containing eukaryotic genes

DATE-ISSUED: February 8, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Robinson; Douglas H.	Washington	DC	20037	

APPL-NO: 8/ 719367 [PALM]

DATE FILED: September 25, 1996

## PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of application Ser. No. 08/261,977, filed on Jun. 17, 1994.

INT-CL: [6] C12 Q 1/04, C12 N 5/16, C12 N 5/08

US-CL-ISSUED: 435/252.3; 435/34, 435/71.1, 435/240.2, 435/252.1

US-CL-CURRENT: 435/252.3; 435/252.1, 435/34, 435/71.1

FIELD-OF-SEARCH: 435/240.2, 435/34, 435/252.1, 435/252.3, 435/71.1

PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

Search Selected

Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/>	<u>4695543</u>	September 1987	Sloma et al.	
<input type="checkbox"/>	<u>4868111</u>	September 1989	Bujard et al.	

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
9012867	November 1990	WOX	

## OTHER PUBLICATIONS

Glover et al. The Canada Lancet &amp; Practitioner, Feb. 1926 pp. 49-62.

Weiss et al. ed. "RNA Tumor Viruses", CSH, 1985, p. 357-408.

Barron's Law Dictionary 3rd Ed., Steven H. Gifis, 1991, Barron's Educational Series, Inc., New York, p. 130.

Webster's II New Riverside University Dictionary, New Riverside Publishing Co., 1988.

Seibert et al. (A) J. of the Reticuloendothelial. Soc. 21(4):279-283, 1977.  
Macomber et al. Med. Hypothesis (England) 32(1):1-9, 1990.  
Seibert et al, (B) Annals New York Academy of Sciences 141:175-201, 1967.  
Seibert et al. (C) Transactions New York Academy of Sciences series 11, vol. 34:  
504-532, 1972.  
Livingston et al. Ann. N.Y. Acad. Sci. vol. 174:636-654, 1970.  
Diller et al. Ann. N.Y. Acad. Sci. vol. 174:655-674m 1970.  
Acevedo et al. J. Gen. Microbiol. 133:783-791, 1987.  
Inoue et al. Nature 205:408-409, 1965.  
Mattman et al. "Characteristics of Filterable Forms," Cell Wall Deficient Forms,  
Stealth Pathogens, chap 24 2nd Edit. pp. 209-216, 289-294, 311-321, 1993.  
Nuzum et al. Symposium on Cancer Before Clinical Congress of American College of  
Surgeons, New York, Oct. 20-26, 1924, pp. 343-352.

ART-UNIT: 163

PRIMARY-EXAMINER: Eisenschenk; Chris

ASSISTANT-EXAMINER: Zeman; Manyk

ATTY-AGENT-FIRM: Rothwell, Figg, Ernst & Kurz, p.c.

#### ABSTRACT:

Bacteria containing eukaryotic and/or viral genes, and often having highly pleiomorphic morphology, are obtained by culturing virally-infected eukaryotic cells under aseptic, low oxygen conditions. The bacteria so produced express products encoded by the eukaryotic genes. Analyses indicate that several isolates obtained from culturing retrovirally-infected human brain capillary endothelial cells express human-specific genes previously mapped to widely separated human chromosomes.

14 Claims, 0 Drawing figures

## Print Request Result(s)

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**Printer Name: cm1\_9e12\_gbefptr**  
**Printer Location: cm1\_\_9e12**

- US006022730: Ok

L11 ANSWER 2 OF 2 MEDLINE  
ACCESSION NUMBER: 91177643 MEDLINE  
DOCUMENT NUMBER: 91177643 PubMed ID: 2007536  
TITLE: Primary clear cell carcinoma of the endometrium: a  
clinicopathologic study of 20 cases.  
AUTHOR: Kanbour-Shakir A; Tobon H  
CORPORATE SOURCE: University of Pittsburgh School of Medicine,  
Pennsylvania.  
SOURCE: INTERNATIONAL JOURNAL OF GYNECOLOGICAL PATHOLOGY, (1991)  
10

(1) 67-78.  
Journal code: GR8; 8214845. ISSN: 0277-1691.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199104  
ENTRY DATE: Entered STN: 19910519  
Last Updated on STN: 19910519  
Entered Medline: 19910429

AB Primary clear cell adenocarcinoma of the endometrium (CCE) is a rare, aggressive tumor, representing 1-5.5% of all primary endometrial cancers. Twenty cases of CCE were studied, covering the period 1973-1987. Both endometrial curettings and hysterectomy with bilateral salpingo-oophorectomy (BSO) specimens were reviewed. Treatment was total abdominal hysterectomy/BSO for all patients with/without pre- and postoperative and/or post-operative chemotherapy. Grossly the tumors formed fleshy, soft masses and involved most of the endometrial surface. The tumor arose in part in an endometrial polyp in 10 cases. Myometrial penetration was found in 12 cases and varied from 5 to 100%. The neoplasm exhibited the following microscopic patterns in pure form or mixed: papillary, tubulocystic, glandular, and solid. All cases were graded as **poorly differentiated (grade 3)** adenocarcinomas. The stroma surrounding tumor cells showed a lymphoplasmocytic cellular infiltrate in all cases. Follow-up varied from 5 to 165 months, with a crude survival of 60%; eight patients died; six

of

those had myometrial invasion of 40-100%. In conclusion, CCEs are specific

tumors with defined histologic parameters in which the cytologic grade and/or tumor morphology do not appear to influence outcome. On the other hand, the depth of myometrial invasion and **clinical staging** are reliable prognostic elements.

**WEST**

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L22: Entry 1 of 6

File: USPT

Jan 30, 2001

DOCUMENT-IDENTIFIER: US 6180404 B1

TITLE: Cultural medium for maintaining neural cells in ambient atmosphere

DATE FILED (1):19990614Other Reference Publication (5):C. Ward Kischer, A. Leibovitz and J. Pindur, The use of a transport medium (L15M15) for bulk tissue storage and retention of viability, Cytotechnology, 2: 181-185, 1989.

**WEST**

Generate Collection

Print

L17: Entry 12 of 16

File: USPT

Jul 22, 1997

DOCUMENT-IDENTIFIER: US 5650096 A

TITLE: Cationic amphiphiles for intracellular delivery of therapeutic molecules

Detailed Description Paragraph Right (63):

A CFT-1 cell line (human cystic fibrosis bronchial epithelial cells immortalized with papillomavirus) provided by Dr. James Yankaskas, University of North Carolina, Chapel Hill, was used for the in vitro assay. The cells were cultured in Hams F12 nutrient media (Gibco/BRL No. 31765-027) supplemented with 2% fetal bovine serum ("FBS", Irvine Scientific, No. 3000) and 7 additional supplements. Cells were then plated into 96-well tissue culture plates at a density of approximately 7,500 cells/well. Before being used in the assay, cells were allowed to grow for periods of 5-7 days until a confluent pattern had been achieved.

**WEST**

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L17: Entry 2 of 16

File: USPT

Nov 27, 2001

DOCUMENT-IDENTIFIER: US 6323191 B1

TITLE: Small molecule chloride transport

Detailed Description Paragraph Right (43):

The tracheobronchial surface epithelial cell line (CFT1) was generated from a CF (.DELTA.F 508) patient and characterized by Dr. Yankaskas et al. at the University of North Carolina at Chapel Hill. The cells were cultured as described previously. (Yankaskas et al., Am. J. Physiol., 264, C1219-1230 (1993)) Briefly, CFT1 cells were seeded onto 12-well cell culture plates at a density of 50,000 cells/cm.<sup>sup.2</sup> and cultured with Ham's F12 medium supplemented with 2% fetal bovine serum, 5 .mu.g/ml insulin, 3.7 .mu.g/ml endothelial cell growth supplement, 25 ng/ml epidermal growth factor, 30 nM triiodothyronine, 1 .mu.M hydrocortisone, 5 .mu.g/ml transferrin, and 10 ng/ml cholera toxin (Gibco).

**WEST**

Generate Collection

Print

L11: Entry 1 of 31

File: USPT

Apr 9, 2002

DOCUMENT-IDENTIFIER: US 6368789 B1

TITLE: Screening methods to identify inhibitors of telomerase activity

DATE FILED (1):

19950605Detailed Description Paragraph Right (113):

In another method ascitic fluid cells were collected and washed as described above. The cellular pellet was resuspended in a-MEM with 10% fetal calf serum and cultured in 150 mm dishes. After 12 hours the media was removed and new plates were used to separate the adhering fibroblasts from the non-adhering cells in the medium. After 12 hours the media containing mostly tumor clumps was removed from the second plates and allowed to adhere in DMA F12 medium supplemented with 3% fetal calf serum, 5 ng/ml EGF, 5 .mu.g/ml insulin, 10 gg/ml human transferrin, 5.times.10.sup.-5 M phosphoethanolamine and 5.times.10.sup.-5 M ethanolamine. These tumor cells were cultured for DNA analysis and S100 extracts.



# WEST Search History

DATE: Saturday, April 20, 2002

<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
side by side		result set	
DB=JPAB,EPAB,DWPI; PLUR=NO; OP=ADJ			
L25	L23 and medium adj 199	4	L25
L24	L23 and F12	67	L24
L23	(endometrial or explant\$1 or epithelial or adenocarcinoma\$1 or tumor or tumour)	36738	L23
DB=USPT; PLUR=NO; OP=ADJ			
L22	L21 and @ad<20010328	6	L22
L21	L20 with transport\$3	6	L21
L20	Leibovitz or (l-15)	659	L20
L19	L18 and @ad<20010328	15	L19
L18	L13 and (endometrial or explant\$1 or epithelial or adenocarcinoma\$1 or tumor or tumour)	15	L18
L17	L16 same (endometrial or explant\$1 or epithelial or adenocarcinoma\$1 or tumor or tumour)	16	L17
L16	l13 or l15	129	L16
L15	L12 with F12	48	L15
L14	L13 same F12	0	L14
L13	L12 with l1	81	L13
L12	(1% or 2% or 3% or 4%) adj4 serum	6946	L12
L11	L10 and @ad<20010328	31	L11
L10	L9 same (endometrial or explant\$1 or epithelial or adenocarcinoma\$1 or tumor or tumour)	31	L10
L9	l6 or l8	307	L9
L8	L4 with f12	148	L8
L7	L6 with F12	0	L7
L6	L4 with l1	159	L6
L5	("less than" 5%) adj4 serum	0	L5
L4	(1% or 2% or 3% or 4% or "<5%") adj4 serum	10315	L4
L3	L2 same serum	15	L3
L2	L1 same F12	18	L2
L1	medium adj 199	1081	L1

END OF SEARCH HISTORY

L9 ANSWER 20 OF 29 MEDLINE DUPLICATE 11

ACCESSION NUMBER: 88226943 MEDLINE

DOCUMENT NUMBER: 88226943 PubMed ID: 3131248

TITLE: Model for invasion of human tissue culture cells by  
Neisseria gonorrhoeae.

AUTHOR: Shaw J H; Falkow S

CORPORATE SOURCE: Department of Medical Microbiology, Stanford University  
School of Medicine, California 94305.

CONTRACT NUMBER: 1P01A21912

SOURCE: INFECTION AND IMMUNITY, (1988 Jun) 56 (6) 1625-32.  
Journal code: GO7; 0246127. ISSN: 0019-9567.

PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198806

ENTRY DATE: Entered STN: 19900308  
Last Updated on STN: 19900308  
Entered Medline: 19880630

AB A tissue **culture** model has been developed for studying the  
ability of Neisseria gonorrhoeae to invade eucaryotic cells. The cell  
line

HecIB, a human adenocarcinoma **endometrial** cell line, was found  
to support gonococcal invasion. The bactericidal antibiotic  
**gentamicin** was used to kill those bacteria that had not entered  
the HecIB cells, allowing us to quantitate internalized bacteria. Kinetic  
studies showed an increase in the titer of **gentamicin**-protected  
gonococci at 4 h postinfection followed by a decrease; a second increase  
occurred after 6 h. The state of piliation did not affect the degree of  
invasion when the bacteria were spun down onto the monolayer. Gonococcal  
invasion was inhibited when the HecIB cells were preincubated with  
cytochalasin D before bacterial infection. N. lactamica was used as a  
negative control. No internalized N. lactamica cells were observed by  
electron microscopy. Electron microscopy documented the intracellular  
location of the gonococci in HecIB cells and the eventual destruction of  
the invaded HecIB cells. After 24 h, clusters of gonococci encased in a  
matrix of cell debris were observed.

completely abolished interleukin-1 alpha-induced cyclooxygenase-2 messenger ribonucleic acid expression, suggesting that the cytokine caused

transcriptional activation of the cyclooxygenase-2 gene. Experiments were conducted to examine whether interleukin-1 receptor antagonist could suppress interleukin-1-induced cyclooxygenase-2 expression. Cells were preincubated for 30 minutes with interleukin-1 receptor antagonist and then challenged with interleukin-1 alpha. Northern and Western analyses revealed that interleukin-1 receptor antagonist blocked interleukin-1 alpha-induced expression of cyclooxygenase-2 messenger ribonucleic acid transcripts and the subsequent appearance of cyclooxygenase-2 protein. Interleukin-1 receptor antagonist had no effect on the constitutive expression of cyclooxygenase-1 messenger ribonucleic acid and protein. Interleukin-1 receptor antagonist failed to alter prostaglandin E2 formation in response to tumor necrosis factor-alpha, indicating that the antagonist is specific for interleukin-1 family cytokines. Finally, interleukin-1 receptor antagonist acted as a partial agonist in some experiments in that relatively high concentrations (> 100 ng/ml) caused a modest increase in prostaglandin E2 and F2 alpha production. CONCLUSIONS: These data indicate that interleukin-1 receptor antagonist is a potent inhibitor of interleukin-1-induced arachidonic acid metabolism and could possibly serve as an endogenous or exogenous modulator of interleukin-1 action in the **endometrial** epithelium.

L5 ANSWER 29 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1992:2895 BIOSIS

DOCUMENT NUMBER: BA93:2895

TITLE: CACO-2 **CELLS CULTURED** IN SERUM-FREE  
MEDIUM AS A MODEL FOR THE STUDY OF ENTEROCYTIC  
DIFFERENTIATION IN-VITRO.

AUTHOR(S): JUMARIE C; MALO C

CORPORATE SOURCE: MEMBRANE TRANSPORT RES. GROUP, DEP. PHYSIOL., FAC. MED.,  
UNIV. MONTREAL, MONTREAL, QUE., CAN. H3C 3J7.

SOURCE: J CELL PHYSIOL, (1991) 149 (1), 24-33.

CODEN: JCLLAX. ISSN: 0021-9541.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB Caco-2 cells, which express spontaneous enterocytic differentiation at confluency, is one of the most relevant in vitro models for the study of differentiation and regulation of intestinal functions. However, these **cells** are normally **cultured** in the presence of 15-20% serum which renders extremely complex the identification of the factors involved in the regulation of both proliferation and differentiation.

This study has been devoted to the establishment of chemically defined culture conditions which can sustain growth and differentiation of Caco-2 cells. The replacement of serum by ITS (insulin, transferrin, and selenium) allowed for normal structural and functional differentiation of cells as revealed by the establishment of cell polarity and the expression of brush-border membrane enzyme markers (sucrase, maltase, lactase, alkaline phosphatase, .gamma.-glutamyl-transferase, aminopeptidase N, and dipeptidyl-dipeptidase IV), although the levels of sucrase activity were lower in ITS-supplemented medium. Coating petri dishes with either type

IV collagen or basement membrane proteins (Matrigel) did not improve the differentiation of cells, brush-border membrane enzyme activities being, in fact, lower when the cells were grown on these substrata. When triiodothyronine (T3, 5 .times. 10<sup>-8</sup> M) was added to the ITS-supplemented medium, disaccharidase and alkaline phosphatase activities were significantly increased while .gamma.-glutamyltransferase activity was diminished by T3 and stimulated by epidermal growth factor (1.6 .times. 10<sup>-6</sup> M). On the other hand, hydrocortisone (HC, 10<sup>-6</sup> M) did not modify disaccharidase and peptidase activities. These data clearly show that Caco-2 cells can be maintained in serum-free medium and that this system allows the study of the factors involved in the regulation of the differentiation of enterocyte in vitro.

L5 ANSWER 12 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1997:512230 BIOSIS

DOCUMENT NUMBER: PREV199799811433

TITLE: Control of cell cycle regulatory protein expression by 1,25-dihydroxyvitamin D-3 in human promyelocytic HL-60 leukemic **cells cultured** in serum-free medium.

AUTHOR(S): Laud, Ketan; Hsieh, Tze-Chen; Wu, Joseph M. (1)

CORPORATE SOURCE: (1) Dep. Biochem. Mol. Biol., New York Med. Coll., Basic Sci. Build., Valhalla, NY 10595 USA

SOURCE: International Journal of Oncology, (1997) Vol. 11, No. 5, pp. 1119-1122.  
ISSN: 1019-6439.

DOCUMENT TYPE: Article

LANGUAGE: English

AB 1,25-Dihydroxyvitamin D-3 (herein referred to as vitamin D-3), the natural

vitamin D-3 formed by successive hydroxylation of cholecalciferol at the 25 and 1 alpha position, and numerous vitamin D-3 analogs, have been reported to decrease proliferation and promote terminal differentiation from several types of human malignant cells, including the human promyelocytic HL-60 leukemic cells. The purpose of this study was to determine if and to what extent the **cell culture** conditions affect the sensitivity of the HL-60 cells to vitamin D-3, both in terms of cell growth, differentiation, and changes in expression of specific proteins. Addition of 10 nM and 100 nM vitamin D-3 to HL-60 **cells cultured** in the serum-free, chemically defined medium of **insulin/transferring selenium** (ITS) effected cell growth differently than cells maintained in a fetal bovine serum-supplemented medium. In addition to the greater degree of growth suppression by 100 nM vitamin D-3, cells maintained in serum-free medium also displayed significantly higher levels of monocytic differentiation. Furthermore, Western blot analysis showed that a pronounced arrest of cell cycling at the G-1-to-S-phase transition, concomitant with a corresponding 36% down-regulation of cyclin D1 and, in parallel, a similar decreased hyperphosphorylation of pRb, was elicited

by

100 nM vitamin D-3. These results indicate that the sensitivity of HL-60 cells to vitamin D-3 is dependent on the availability of serum.

L5 ANSWER 38 OF 56 MEDLINE

ACCESSION NUMBER: 87244043 MEDLINE

DOCUMENT NUMBER: 87244043 PubMed ID: 3297308

TITLE: Growth and continuous passage of COMMA-D mouse mammary epithelial cells in hormonally defined serum-free medium.

AUTHOR: Riss T L; Sirbasku D A

CONTRACT NUMBER: CA-26617 (NCI)

CA-38024 (NCI)

SOURCE: CANCER RESEARCH, (1987 Jul 15) 47 (14) 3776-82.

Journal code: CNF; 2984705R. ISSN: 0008-5472.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198708

ENTRY DATE: Entered STN: 19900305

Last Updated on STN: 20000303

Entered Medline: 19870820

AB Growth of the mouse mammary epithelial cell line designated COMMA-D has been studied in serum-free medium (SFM) formulated with Ham's F12 and Dulbecco's modified Eagle's medium (1/1) containing 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 2 mM glutamine, gentamicin (50 micrograms/ml; basal medium) and supplemented with insulin (10 micrograms/ml), transferrin (10 micrograms/ml), selenous acid (10 ng/ml), epidermal growth factor (20 ng/ml; EGF), 10 nM 3,5,3'-triiodothyronine, 50 microM ethanolamine, 1.0 nM 17

beta-estradiol,

65 microM glutathione, and ovalbumin (100 micrograms/ml). COMMA-D cells were able to undergo serial passage and continued to exhibit dome formation after 20 passages in SFM. Cells seeded at low density in SFM underwent four population doublings at low passage number in 1 week compared to six doublings for cells grown in medium containing **insulin, transferrin, selenium, EGF, and 1% fetal bovine serum**. After many passages in SFM, the growth rates of cells were similar to those in serum-supplemented medium used for stock

culture.

Deletion of insulin or EGF from SFM resulted in cell growth similar to that of cells seeded in basal medium alone. When cells were seeded in basal medium without added supplements, addition of insulin or EGF resulted in 29 and 22%, respectively, of the number of cells grown in SFM for 5 days. However, when insulin and EGF were combined in basal medium, the cell number at 5 days was 83% of that in SFM. When insulin was

deleted

from SFM, COMMA-D cells became responsive to insulin-like growth factors

I

and II. The growth-promoting characteristics of EGF and transforming growth factor alpha were compared in SFM and were not distinguishable, showing identical dose-response curves. When incorporation of [3H]thymidine was used as an assay of cell growth, saturating levels of basic fibroblast growth factor (20 ng/ml) showed a stimulation 1.35 times greater than EGF (20 ng/ml). When EGF and fibroblast growth factor were combined, the stimulation was 1.75 times greater than EGF alone

suggesting

that COMMA-D cells are responsive to multiple classes of growth factors. COMMA-D cells seeded in basal medium supplemented with insulin, transferrin, and selenous acid have been used to detect mitogenic

activity

present in extracts of hypothalamus, uterus, and pituitary. The results show that COMMA-D cells can be grown long term in a hormonally defined serum-free medium and that maximal mitogenic effects were seen only with the addition of two or more growth factors.

L5 ANSWER 30 OF 56

MEDLINE

DUPLICATE 14

ACCESSION NUMBER: 91035144 MEDLINE

DOCUMENT NUMBER: 91035144 PubMed ID: 2121705

TITLE: Characterization of differentiated Syrian golden hamster pancreatic duct cells maintained in extended monolayer culture.

AUTHOR: Hubchak S; Mangino M M; Reddy M K; Scarpelli D G

CORPORATE SOURCE: Department of Pathology, Northwestern University Medical School, Chicago, Illinois 60611.

CONTRACT NUMBER: CA34051 (NCI)

SOURCE: IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY, (1990 Sep) 26 (9) 889-97.

Journal code: HEQ; 8506951. ISSN: 0883-8364.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199012

ENTRY DATE: Entered STN: 19910208

Last Updated on STN: 19910208

Entered Medline: 19901204

AB Epithelial cells isolated from fragments of hamster pancreas interlobular ducts were freed of fibroblast contamination by plating them on air-dried collagen, maintaining them in serum-free Dulbecco's modified Eagle's (DME):F12 medium supplemented with growth factors, and selecting fibroblast-free aggregates of duct cells with cloning cylinders. Duct epithelial cells plated on rat type I collagen gel and maintained in DME:F12 supplemented with Nu Serum IV, bovine pituitary extract, epidermal

growth factor, 3,3',5-triiodothyronine, dexamethasone, and **insulin**, **transferrin**, **selenium**, and linoleic acid conjugated to bovine serum albumin (ITS+), showed optimal growth as monolayers with

a

doubling time of about 20 h and were propagated for as long as 26 wk. Early passage cells consisted of cuboidal cells with microvilli on their apical surface, complex basolateral membranes, numerous elongated mitochondria, and both free and membrane-bound ribosomes. Cells grown as monolayers for 3 mo. were more flattened and contained fewer apical microvilli, mitochondria, and profiles of rough surfaced endoplasmic reticulum; in addition, there were numerous autophagic vacuoles. Functional characteristics of differentiated pancreatic duct cells which were maintained during extended monolayer culture included intracellular levels of carbonic anhydrase and their capacity to generate cyclic AMP (cAMP) after stimulation by  $1 \times 10^{-6}$  M secretin. From 5 to 7 wk in culture, levels of carbonic anhydrase remained stable but after 25 to 26 wk decreased by 1.9-fold. At 5 to 7 wk of culture, cyclic AMP increased 8.7-fold over basal levels after secretin stimulation. Although

pancreatic

duct **cells cultured** for 25 to 26 wk showed lower basal levels of cAMP, they were still capable of generating significant levels of cAMP after exposure to secretin with a 7.0-fold increase, indicating that secretin receptors and the adenyl cyclase system were both present and functional. These experiments document that pancreatic duct monolayer cultures can be maintained in a differentiated state for up to 6 mo. and suggest that this culture system may be useful for in vitro physiologic and pathologic studies.